International Journal of Agricultural Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 4, Issue 3, Jun 2014, 11-26

IMPACT OF PGPR AND INORGANIC FERTILIZATION ON GROWTH AND PRODUCTIVITY OF SWEET ANANAS MELON

ABD EL-AAL, M. M. M[1] & H. M. ABD EL-RAHMAN[2]

[1]Department of Agric. Botany, Faculty of Agric, Benha University, Moshtohor, Kaliobya, Egypt

[2]Department of Microbiology, Agric. Botany, Faculty of Agric, Benha University, Moshtohor, Kaliobya, Egypt

ABSTRACT

This study was carried out at experimental station in Faculty of Agriculture, Moshtohor, Benha University, Egypt during 2011 and 2012 seasons to improve the morphological and physiological characteristics and yield of sweet ananas melon towards maximizing its growth and productivity using plant growth promoting rhizobacteria (PGPR) in combination with inorganic fertilization and to reduce the using of chemical fertilization for vegetables production.

Obtained results could be summarized as the following: the dual treatments of bio and inorganic fertilization gave higher parameters of vegetative growth, photosynthetic pigments content, total fruits yield/ plant and nutritional status compared with the solely fertilization ones. The best results of vegetative growth , photosynthetic pigments content total fruiting/ plant and chemical composition of leaves and fruits were obtained with the application of biofertilizer + full chemical fertilization dose followed by PGPR + 3/4 chemical fertilization without any significant differences. PGPR treatments reduced dose of chemical fertilization and decrease the environmental pollution caused by repeated application of inorganic fertilizers. The role of phytohormones like substances produced PGPR in increasing no. of pistilate flowers/ plant in order to yield was investigated.

KEYWORDS: PGPR, Inorganic Fertilization, Sweet Ananas Melon, Vegetative Growth, Fruits Yield and PGPR

INTRODUCTION

Melon (*Cucumis melo* L., family Cucurbitaceae) is a commercially an important horticultural vegetable fruit crop cultivated in temperate, subtropical and tropical regions worldwide. Melon was domesticated in the eastern Mediterranean region

Melon fruit enjoys widespread popularity among consumers owing to its taste and high nutritional value, **Majkowska-Gadomska (2009**). The melon fruits are naturally low in fat and sodium, have no cholesterol and provide many essential nutrients such as potassium, in addition to being a rich source in beta-carotene, vitamin C and a good source of carbohydrates, **Lester (1997**).

Research on fruits and vegetables is increasing because of their benefits to human health. Indeed, a positive correlation has been reported between fruit consumption and the decrease risk of several chronic diseases including, cardiovascular obesity disease, and certain types of cancer **Boeing, *et al.*, (2012) and Jansen, *et al.*, (2011**). Excessive and imbalanced use of chemical fertilizers has adversely affected the soil, causing decrease in organic carbon, reduction in microbial flora of soil, increasing acidity and alkalinity and hardening of soil. Increasing the use of chemical fertilizer led

to high cost in vegetable production and creates pollution of their agricultural environment as well as affects the soil fertility; therefore, it has become essential to use untraditional fertilizers as supplements or substitutes for chemical nitrogen fertilizers **Rajasekaran *et al.*, (2012**).

Recently, under Egyptian conditions a great attention is being devoted to reduce the high rates of inorganic fertilizers, the cost of production and environmental pollution via reducing doses of nitrogenous and phosphorus fertilizers by using bio fertilized farming system **El-Habbasha *et al.*, (2007) and El- Nagdy *et al.*, (2010).**

Biological fertilization of non-legume crops by N_2-fixing bacteria had a great importance in recent years. The effect of inoculation had marked influence on the growth of plant, which was reflected to increase yield. This increase might be due to the effect of nitrogen, which was produced by bacteria species, in addition of some growth regulators like IAA and GA3 which stimulated growth. Some bacteria called plant growth promoting rhizobacteria (PGPR), stimulate plant growth **Kapulnik (1991)** and **Kloepper *et al.*, (1991)**. The stimulatory effects of microorganisms may result from either direct or indirect action. Direct effects include production of phytohormones **Noel *et al.*, (1996)** enhancement of availability of some inorganic **Kapulnik (1991)** liberation of phosphates and micronutrients, nonsymbiotic nitrogen fixation and stimulation of disease-resistance mechanisms **Lazarovits** and **Nowak (1997)**. Indirect effects arise from PGPR altering the root environment and ecology **Glick (1995)**. For example, acting as biocontrol agents and reducing diseases, liberation of antibiotic substances that kill noxious bacteria **Lazarovits** and **Nowak (1997)**. Moreover, it was found that the application of phosphate dissolving bacteria as a bio fertilizer resulted in a reduction of soil pH which increased the solubility of some nutrients such as P, Fe, Zn, Mn and Cu which in turn increased nutrient uptake by plants **Saber** and **Kabesh (1990)**.

Biofertilizers from microorganisms can replace chemical fertilizers to increase crop production. In principle, biofertilizers are less expensive and are more environmentally friendly than chemical fertilizers.

Investigation on the relationship between roots and microbiota are essential to achieve innovations in agriculture and biotechnology. Plant growth promoting bacteria (PGPB) are groups of bacteria which as effective as pure chemical on plant growth enhancement and disease control. PGPB that colonize the root and rhizosphere have met with great success in improving plant growth. A large number of PGPB genera on one hand and rhizobia and few endophytes on the other promise benefit to crop ecosystem for sustainable agriculture. In relation to plant health, the exploitation of such beneficial bacteria may improve agriculture system with economically sound production of human food **Maheshwari (2010**)

Integrated nutrient management (INM) is a well-accepted approach for the sustainable management of soil productivity and increased crop production **FAO (2008**). Therefore, the aim of this investigation study the effect of integrated fertilization with bio- and inorganic fertilizers to reach the lowest level of inorganic fertilizers, which does not significantly affect the yield and quality of ananas melon.

MATERIALS AND METHODS

Experimental Design

This study was carried out during two successive seasons of 2011 and 2012 at the Experimental Farm of the Faculty of Agriculture, Moshtohor, Benha University, Egypt to study the effect of PGPR and inorganic fertilizers as well their interactions on the morphological and physiological characteristics i.e., growth parameters, fruit yield and its components of sweet ananas melon (*Cucumis melo* var. *reticulatus*) cv. Rodin hybrid, which locally in Egyptian

markets have a commonly name known as ‘Ananas’ grown in clay soil . Sweet ananas melon were sown at the first week of March in the two growing seasons. Seeds of sweet ananas melon were inoculated by coating with a solution consist of PGPR cultures and 40 % sucrose solution and then took place in the permanent field. The experimental treatments were arranged in a randomized completely blocks design (RCBD) and included six treatments with four replicates as the following.

1	**Inorganic fertilizers (full dose of N, P and K)**
2	**PGPR**
3	**PGPR + 1/ 4 dose of inorganic fertilizers**
4	**PGPR + 1/ 2 dose of inorganic fertilizers**
5	**PGPR +3/ 4 dose of inorganic fertilizers**
6	**PGPR + full dose of inorganic fertilizers**

PGPR solution was containing 500 ml of N_2- fixing free living bacterial cultures (*Azotobacter chroococcum;* 8.4×10^{11} CFU ml^{-1} and *Azospirillium lipoferum* D178; 7.2×10^{11} CFU ml^{-1}) and 500 ml of phosphate dissolving bacterial culture (*Bacillus megaterium;* 8.3×10^{11} CFU ml^{-1} and *Pseudomonas. fluorescens; 9*$\times10^{11}$**)**. The PGPR cultures were prepared by strains reserved in the Agric. Botany Department (Microbiology Branch), Faculty of Agriculture, Benha University, Egypt. Plant growth promoting substances **(PGPs)** production of abovementioned strains tabulated in Table 1.

Table 1: Indole Acetic Acid, Gibberellins and Cytokinins Production by Plant Growth Promoting Rhizobacteria (PGPR) Strains in Vitro

	IAA µg/Ml	Gibberellins (As GA3) µg/Ml	Cytokinins (As Zeatin) µg/Ml
B. megaterium	52.5	765.2	98.5
Ps. Fluorescens	53.9	209.1	123.8
Azo.chroococum	71.8	146.7	107.3
A. lipoferum D178	43.6	157.6	100.8

The PGPR solution is divided into two parts. The first one was added during soil preparation with compost and the second is coated sweet ananas melon seeds except the chemical fertilization treatment, the boost dose of PGPR was added after 30 and 60 days of sowing. Nitrogen, phosphorus and potassium fertilizers (recommended dose) were added as NH_4NO_3 (33.5 % N), Ca $(H_2PO_4)_2.CaCO_3$ (16% P_2O_5) and K_2SO_4 (48 % K_2O). The phosphate fertilizers were added to each experimental plot during soil preparation. Meanwhile, NH_4NO_3 and K_2SO_4 fertilizers were added on two batches. Nitrogen (23 kg N $fed.^{-1}$), fertilizer was added based on experimental treatments. All cultural practices for growing ananas melon were performed as recommended by Egyptian Ministry of Agriculture.

Measurements of Microbial Enzymes Activity

The enzyme activity of dehydrogenase (DH) and alkaline phosphatase (AlP) were measured using method of **Schinner, *et al.*, (1997)** before cultivation and after 15, 35, 50 and 70 days from sowing in soil rhizosphere.

Morphological Characters

Ten plants from each treatment were randomly taken to evaluate vegetative growth and yield characteristics, Many botanical and physiological characteristics were measured and calculated at 65 days after sowing (i.e. the time of flowering), the following characteristics were inspected:

- Stem length (cm.).
- Stem diameter (cm.) at the first inter node.
- Number of branches/plant.
- Number of leaves/plant.
- Fresh and dry weight of stems and leaves.
- Total leaf area (cm^2)/ plant following the method described by **Deriaux , *et al.*, (1973).**
- Specific leaf weight (mg/cm^2) (S.L.W.) was expressed as the amount of leaf dry matter (mg) produced per unit of leaf area (cm^2) according to **Wareing and Phillips (1981)** using the following equation:

$$S.L.W. = \frac{\text{Total leaves dry weight (mg)/plant}}{\text{Total leaf area (cm}^2\text{)/ plant}}$$

Photosynthetic Pigments

Chlorophyll a, b and carotenoids were colorimetrically determined in leaves at 65 days after sowing according to the methods described by **Wettstein (1957)** and then calculated as mg/g fresh weight.

Flowering Characters

- **Total number of Pistilate Flowers / Plant:** Were recorded for each treatment through the two seasons.
- **Abscission Percentage:** Was Calculated According to the Equation

$$\textbf{Abscission \%} = \frac{\textbf{No. of pistilate flowers/plant - No. of fruits/plant}}{\textbf{No. of pistilate flowers / plant}} \times 100$$

Fruits Setting Percentage: Was Calculated According to the Following Equation

$$\textbf{\% of Setted Fruits} = \frac{\textbf{No. of fruits / plant}}{\textbf{No. of pistilate flowers / plant}} \times 100$$

Fruit Yield and Yield Components

Data of the all pickings in the two seasons were used to calculate the following:

- Total Number of fruits/ plant.
- Total yield weight (gm)/ plant

Chemical Analysis

The following determinations in 2012 season were conducted in dry matter of leaves and fruits and calculated as mg/g dry weight.

- **Total Nitrogen** was determined by using wet digestion according to **Piper (1974)** and using microkildahl as described by **Horneck and Miller (1998)**
- **Phosphorus** was determined colorimetrically according to the method of **Sandell (1950)**

- **Potassium** was determined by Flame Photometer Model Carl-Zeiss according to the method of **Horneck and Hanson (1967).**
- **Total Sugars Content** was determined by method described by **A. O. A. C. (1990).**
- **Total Soluble Solids** was measured in juice of the fruits by using a hand Refract meter and calculated as percentages.
- **Endogenous Phytohormones** were determined quantitatively in sweet ananas melon shoots at 65 days after sowing during 2012 season. The method of **Koshioka, *et al.*, (1983)** was used for the HPLC "high – performance liquid chromatography" determination of auxin (IAA), gibberellic acid (GA3) and abscisic acid (ABA). cytokinins were determined by UPLC according to **Nicander, *et al.*, (1993).**

Soil Analyses

Random soil samples were taken before sowing for biological, chemical and physical analysis using **Chapman and Pratt (1961)** and **Jackson (1965)** methods.

Table 2: Physical and Chemical Analyses of the Experimental Soil before Sowing

Soil Texture					EC	O.M (%)	$Caco_3$ (%)
Sand (%)	Silt (%)	Clay (%)	Textural Class	PH	(Ds M^{-1})		
24.4	24.6	51	Clay	7.9	2.16	1.41	1.53

Soil Available Macronutrients (Mg Kg^{-1})			Total Content of Soil Trace Elements (ppm)						
N	P	K	B	Zn	Mn	Cu	Cd	Ni	Pb
22.5	9.1	120	15.15	89.73	935	64.65	0.154	60.56	9.16

Statistical Analysis

Data of morphological, flowering and yield characteristics were statistically analyzed and the means were compared using the least significant difference test (L.S.D) at 5% level according to **Snedecor and Cochran (1982).**

RESULTS AND DISCUSSIONS

Soil Microbial Enzyme Activities

PGPR tended to be stronger effects when applicated with inorganic fertilizer. The combination of PGPR and inorganic fertilizer recorded the higher values of DH and AlP activities comparing with each one separately (**Tables 3, 4**). In all treatments, the DH and AlP activities were increased from the beginning to reach the highest values after 50 days from sowing. That means in the beginning microbial enzyme activity did not active enough and this may be due to the small plant age that accompanied by a few root exudates. After 50 days from sowing, the activity reached the maximum values, where this may be due to the enough nutrients from root exudates and root debris that represent nutritional substances for different soil microorganisms and effect of inorganic fertilization.

Moreover, it could be due to effect of boost inoculation of PGPR in supporting its colonization on plant root. After 70 days from sowing, the activity decreased again, where this may be due to the shortage of nutrients in the rhizosphere or the complete decomposed organic fertilizer or the effect of rhizosphere on soil microbial enzyme activities.

Moreover, intracellular enzyme activities are short-lived because they are degraded by proteases unless they are adsorbed by clays or immobilized by humic molecules ***Burns and Dick*** **(2002**).

Table 3: Effect of Bio- and Chemical-Fertilization on Dehydrogenase Activity in Soil Cultivated with Sweet Ananas Melon

Treatments	Dehydrogenase (µg TPF G^{-1} Dw H^{-1})			
	15 Days	35 Days	50 Days	70 Days
Full Dose of Inorganic Fertilizers	25	35	46	18
PGPR	38	42	58	34
PGPR + 1/ 4 Dose of Inorganic Fertilizers	43	44	61	35
PGPR + 1/ 2 Dose of Inorganic Fertilizers	44	51	64	43
PGPR +3/ 4 Dose of Inorganic Fertilizers	50	67	74	44
PGPR + Full Dose of Inorganic Fertilizers	67	70	77	67

Table 4: Effect of Bio and Chemical-Fertilization on Phosphatase in Soil Cultivated with Sweet Ananas Melon

Treatments	Alkaline Phosphatase (µg *P*np G^{-1} H^{-1})			
	15 Days	35 Days	50 Days	70 Days
Full Dose of Inorganic Fertilizers	12.0	17.7	20.0	14.6
PGPR	16.8	19.3	21.5	15.1
PGPR + 1/ 4 Dose of Inorganic Fertilizers	19.7	21.8	21.8	17.6
PGPR + 1/ 2 Dose of Inorganic Fertilizers	22.0	23.1	26.4	20.7
PGPR +3/ 4 Dose of Inorganic Fertilizers	23.4	27.3	30.1	21.8
PGPR + Full Dose of Inorganic Fertilizers	24.0	31.3	32.6	23.4

The higher records of DH and AlP activities were achieved by using the inorganic fertilizers and PGPR together compared with inorganic fertilizers only. These results observed the role of inoculation in proliferation of microorganism in rhizosphere besides improving the microbiological activity in the rhizosphere **Kohler *et al.*, (2007**)

Inorganic fertilizers had relatively less effect on soil microbial biomass and activities than organic fertilizers **Parham *et al.*, (2003) and Plaza *et al.*, (2004**)

Activity of phosphatase is important in studying the phosphorus cycle because this can provide a route for P inorganicization and plant uptake. However, similarity in their activities was not persistent, and sometimes even contrasting. The significant greater activities of alkaline phosphatase in the PGPR treated soils may be due to enhance microbial activity ***Mandal, et al.*, (2007).**

Vegetative Growth Characteristics

As shown in **Table (5)** in most cases different applied treatments significantly affected on different estimated vegetative growth characteristics of sweet ananas melon i.e., plant length, stem diameter, no. of branches/ plant, no. of leaves/ plant, stems and leaves fresh and dry weight/ plant, total leaf area / plant and specific leaf weight/plant at 65 days after sowing during 2011 and 2012 seasons.

Table 5: Effect of Inorganic and Bio - Fertilization on Plant Growth Characteristics of Sweet Ananas Melon at 65 Days after Sowing during 2011 and 2012 Seasons

Treatments	Plant Length (cm)	Stem Diameter (cm)	No. of Branches/ Plant	No. of Leaves/ Plant	Stems Fresh Weight (g)/ Plant
Season 2011					
Full dose of inorganic fertilizers	107.4	1.13	5.2	49.3	114.5
PGPR	94.3	1.02	3.9	35.5	79.4
PGPR + 1/ 4 dose of inorganic fert.	101.2	1.13	4.3	47.2	99.3
PGPR + 1/ 2 dose of inorganic fert.	105.8	1.41	4.9	57.7	101.3
PGPR +3/ 4 dose of inorganic fert.	115.7	1.54	5.6	58.6	125.1
PGPR + full dose of inorganic fert.	117.5	1.58	5.8	59.6	131.4
L.S.D. 0.05	5.02	0.22	0.48	6.08	8.42
Season 2012					
Full dose of inorganic fertilizers	110.4	1.05	5.0	52.1	121.4
PGPR	105.7	0.93	4.1	41.6	95.1
PGPR + 1/ 4 dose of inorganic fert.	110.4	1.19	5.1	55.5	120.5
PGPR + 1/ 2 dose of inorganic fert.	116.5	1.13	5.7	61.8	113.3
PGPR +3/ 4 dose of inorganic fert.	128.2	1.62	6.2	67.3	128.8
PGPR + full dose of inorganic fert.	131.0	1.77	6.5	72.2	139.2
L.S.D. 0.05	**4.57**	**0.13**	**0.67**	**5.49**	**11.27**

Treatments	Leaves Fresh Weight(G)/ Plant	Stems Dry Weight(G)/ Plant	Leaves Dry Weight (G)/ Plant	Total Leaf Area (Cm2)/ Plant	Specific Leaf Weight/Plan t (Mg/Cm2)
Season 2011					
Full dose of inorganic fertilizers	194.3	13.4	55.5	1954.5	25.76
PGPR	175.8	11.7	43.7	1683.6	24.88
PGPR + 1/ 4 dose of inorganic fert.	189.4	13.8	49.7	1887.4	25.54
PGPR + 1/ 2 dose of inorganic fert.	219.4	15.5	51.3	1997.5	25.96
PGPR +3/ 4 dose of inorganic fert.	246.2	16.2	57.3	2154.5	27.18
PGPR + full dose of inorganic fert.	253.8	16.3	58.4	2286.8	27.32
L.S.D. 0.05	13.54	2.08	5.21	146.15	0.74
Season 2012					
Full dose of inorganic fertilizers	233.4	16.3	56.4	1881.3	27.56
PGPR	191.7	13.8	43.1	1792.7	22.91
PGPR + 1/ 4 dose of inorganic fert.	221.5	18.7	47.8	2063.7	23.16
PGPR + 1/ 2 dose of inorganic fert.	227.4	14.3	49.4	2179.5	25.88
PGPR +3/ 4 dose of inorganic fert.	269.3	22.8	64.5	2195.4	27.56
PGPR + full dose of inorganic fert.	284.1	24.0	67.0	2120.9	31.59
L.S.D. 0.05	**10.89**	**2.85**	**4.06**	**115.48**	**0.38**

In this respect, the most superior treatments were the using of PGPR and full dose of inorganic fertilizers, PGPR + 3/4 dose of inorganic fertilizers and PGPR + 1/2 dose of inorganic fertilizers, when compared with each one individually in the two seasons. These results are a great interest, because at this stage of growth great simulative positive differences existed with various applied treatments. Since, that could be prolonged to the advanced growth stages including each of flowering and the final fruit yield as well as the high quality of yielded fruits.

In addition, the increase of stem diameter may be accompanied with basic anatomical modification in different stem tissues specially phloem and xylem. Therefore, that could be accompanied with a great variations in the nature of sweet ananas melon branching. Besides, increasing of stem diameter accompanied with increasing the plant height means

that applied treatments lead to vigorous growth and more healthy plant under different used fertilization treatments especially the PGPR in combination with inorganic fertilizers with different doses rather than each one individually.

In this respect, increasing of formed branches on growing plant could be reversed upon many other characters such as number of leaves, leaf area, leaves dry weight, flowering and finally the yielded fruits. Furthermore, increment of shoots (stems & leaves) fresh and dry weight due to increases of number of both branches and leaves and the total leaf area as mentioned, Increment of leaf characteristics (number and area) as well as their content of photosynthetic could be a basic for increasing the photosynthetic efficiency.

Regarding the total leaf area per plant, it also behaved as the same as the above-mentioned characteristics. Since all applied treatments showed high significant increase, its maximum values recorded with the interaction treatments. Increment of leaf area is a great interest because that could be reflected upon the efficiency of photosynthesis by accumulating more assimilates and high rates of their translocation specially toward fruits forming. In addition, it could be noticed that increment of this area was preceded with high number of branches and leaves as well. The present findings are in agreement with those reported by **El-Kramany *et al.*, (2000), El-Habbasha *et al.*, (2007), Hosseny and Ahmed (2009), Kumar *et al.*, (2009), Boghdady *et al.*, (2012) and Dubey *et al.*, (2012).**

Effect of Applied Treatments on Photosynthetic Pigments, Macronutrients and Total Sugars Content of Sweet Ananas Melon Shoot at 65 Days after Sowing

Data presented in **Table (6)** clearly indicated the effect of different applied treatments on chlorophyll A, B, carotenoids, minerals and total sugars concentrations of ananas melon leaves. Regarding the photosynthetic pigments, data revealed that ananas melon inoculated with PGPR in combination with inorganic fertilizer gave the highest values of total leaves content of photosynthetic pigments, (i.e., chlorophyll A & B chl.(A+B) and caro.) at 65 days after sowing comparing with each one individually. In this respect, the most effective treatment which led to maintain the highest concentrations of determined photosynthetic pigments was PGPR + full dose of inorganic fertilizers followed by PGPR + 3/4 dose of inorganic fertilizers and PGPR + 1/2 dose of inorganic fertilizers, respectively comparing with the individual treatments.

In similar trend, inorganic concentration (i.e., N, P and K) and total sugars content were affected as mentioned results. Generally, it could be concluded that different applied treatments were mostly effective, which induced an active internal metabolic case and most effective (i.e., chlorophyll, carotenoids, inorganic and sugars). At the same time, this was accompanied with good morphological, minerals status and agronomical performances. In this respect, the most superior treatments were PGPR + full dose of inorganic fertilizers followed by PGPR + 3/4 dose of inorganic fertilizers and PGPR + 1/2 dose of inorganic fertilizers, respectively. This is might be due to the effect of PGPR on releasing of macronutrients or on the nutrients availability.

Similar data also evidently confirmed the stimulatory and significantly effects of different applied treatments specially inorganic fertilization in combination with biofertilizer treatments comparing with individual ones upon dry matter production and accumulation in leaves and branches. In general, data in **Table (6)** not only being a direct results for that vigorous growth obtained in **Table (5)** but also could be considered an indicator for expectable high yield of fruits. The obtained results are generally in agreement with those reported by **Hosseny and Ahmed (2009), Abdo (2008) and Boghdady *et al.*, (2012).**

Table 6: Effect of Inorganic and Bio – Fertilization on Photosynthetic Pigments, Inorganic and Total Sugars Content of Sweet Ananas Melon Leaves at 65 Days after Sowing during 2012 Season.

Treatments	Photosynthetic Pigments mg/g Fresh Weight				Inorganic (mg/g Dry Weight)			Total Sugars
	Chl. A	Chl. B	Chl A+b	Carote-noids	N	P	K	
Full dose of inorganic fertilizers	0.73	0.61	1.30	0.64	51.4	45.8	28.4	87.5
PGPR	0.69	0.49	1.22	0.53	45.4	39.7	26.5	77.3
PGPR + 1/ 4 dose of inorganic fert.	0.74	0.51	1.25	0.61	39.8	41.5	22.4	81.4
PGPR + 1/ 2 dose of inorganic fert.	0.87	0.69	1.56	0.63	51.6	46.4	27.3	89.0
PGPR +3/ 4 dose of inorganic fert.	0.89	0.76	1.65	0.78	69.5	49.4	37.3	93.2
PGPR + full dose of inorganic fert.	0.93	0.72	1.65	0.71	72.7	50.1	38.6	95.8

Effect of Different Applied Treatments on Endogenous Phytohormones of Sweet Ananas Melon Shoot at 65 Days after Sowing

Data in **Table (7)** showed the changes in endogenous phytohoromones, indole acetic acid (IAA), gibberellic acids (GA3), cytokinins and abscisic acid (ABA) of sweet ananas melon fertilized with PGPR, inorganic fertilizers as well as their interactions. In this respect, PGBR + full dose of inorganic fertilizers followed by PGPR + 3/4 dose of inorganic fertilizers and PGPR + 1/2 dose of inorganic fertilizers, are the most effective treatments which greatly improved the morphological, metabolical performances of sweet ananas melon as obvious from the previously mentioned and discussed results obtained in the present study compared with the individual treatments (i.e., PGPR or full dose of inorganic fertilizers). As for auxin level, it was highly increased in ananas melon shoot with different combinations of bio and inorganic treatments compared with that of solely treatments. Again, PGPR + full dose of inorganic fertilizers was the most effective followed by PGPR + 3/4 dose of inorganic fertilizers and PGPR + 1/2 dose of inorganic fertilizers. With regard to, gibberellins level, data in **Table (7)** clearly showed that the level of gibberellins in sweet ananas melon leaves was behaved as the same as auxins level.

Table 7: Effect of Inorganic and Bio – Fertilization on Endogenous Hormonal Profile in Shoots of Sweet Ananas Melon Plants at 65 Days after Sowing during 2012 Season

Treatments	Promoters				Inhibitors	Promoters/ Inhibitors
	Gibberellins (GA_3) µg/g F.wt.	Auxins (IAA) µg/g F.wt.	Cytokinins µg/g F.wt.	Total Promoters µg/g F.wt.	Abscisic Acid (ABA) µg/g F.wt.	
Full dose of inorganic fertilizers	53.44	32.38	66.26	169.23	1.87	90.50
PGPR	41.51	22.54	42.27	106.32	2.76	38.52
PGPR + 1/ 4 dose of inorganic fert.	65.82	45.86	68.75	180.43	1.53	117.93
PGPR + 1/ 2 dose of inorganic fert.	57.27	47.78	76.58	172.40	1.74	99.08
PGPR +3/ 4 dose of inorganic fert.	61.74	56.38	81.46	199.58	1.27	157.15
PGPR + full dose of inorganic fert.	73.48	65.42	97.08	235.98	1.08	218.50

Furthermore, **Table (7)** clearly indicates that the level of cytokinines positively responded to the different assigned treatments. Since, its lowest case was in the PGPR solely treatment.

Generally, phytohormones those promote growth aspects i.e., growth promoters, auxins, gibberellin and cytokinin were highly increased with different assigned treatments compared with individual PGPR treatment. The treatment of PGPR + chemical fertilization (full dose) gave the highest value of promoting phytohormones level, where the increment

reached more than two times of solely PGPR treatment. Also, increment of endogenous hormones in sweet ananas melon obtained in the present study could be interpret of both the obtained variations in different studied metabolically features **(Tables 7 and 9)**, the improvement of growth features **(Table 5),** flowering and yield **(Table 8)**. For example, increasing cytokinins could be in favor of increasing the number of formed branches and that could also increase transverse growth on the account of longitudinal one as well as increasing of sink organs (i.e., fruits) ability to accumulate and storage more assimilates.

Increasing of endogenous promoting phytohormones content of sweet ananas melon shoot in case of inoculation with PGPR may be due to the beneficial effect of PGPR strains in production of **PGPs (Table 1)**. This may explain the increase of cytokinins and other promoting hormones in response to biofertilizers application in combination with inorganic fertilization treatments.

With regard to the growth inhibitor (abscises acid), its level was reduced with various assigned treatments compared with the solely treatments (i.e., PGPR or full dose of chemical fertilization) but the reduction acid was more obvious with biofertilizers application combined with inorganic fertilization treatments.

Moreover, the proportions of total promoters to the inhibitor abscisic acid **Table (7)** was increased with the different assigned combination of PGPR and inorganic fertilizers treatments compared with each one individually and reached its maximum value with combination of PGPR and full dose of inorganic fertilizers treatment.

In this respect, these results being a great interest for interpreting each of the obtained vigorous growth and the great fruit yield of ananas melon attained in the present study.

It was obvious from data simulative effects of these treatments to enhance the internal metabolically features such as promoting hormones of sweet ananas melon towards maximizing its growth and productivity. The obtained results are almost in harmony with those reported by **Kapulnik, (1991) ; Kloepper *et al.*, (1991) and Noel *et al.*, (1996), Hosseny and Ahmed (2009).**

Effect of Different Applied Treatments on Flowering, Fruits Yield and Bioconstituents of Sweet Ananas Melon Fruits

Data in **Table (8)** showed that flowering characteristics, fruit yield and its components of ananas melon were highly affected by different applied treatments during 2011 and 2012 seasons.

Table 8: Effect of Inorganic and Bio – Fertilization on Flowering and Yield Characteristics of Sweet Ananas Melon during 2011 and 2012 Seasons

Treatments	No. of Pistilate Flowers/ Plant	No. of Setted Fruits/ Plant	% of Abscission	% of Setted Fruits	Weight of Fruit (G)	Fruits Yield (G)/ Plant
Season 2011						
Full dose of inorganic fertilizers	11.7	4.1	64.96	35.04	1169.27	5175.3
PGPR	11.3	3.5	69.03	30.97	1071.86	3751.5
PGPR + 1/ 4 dose of inorganic fert.	11.5	4.7	59.13	40.87	1124.51	5285.2
PGPR + 1/ 2 dose of inorganic fert.	12.7	4.5	64.57	35.43	1168.37	5274.5
PGPR +3/ 4 dose of inorganic fert.	12.7	5.6	61.59	38.41	1233.41	5448.6
PGPR + full dose of inorganic fert.	12.8	5.8	53.97	46.03	1280.80	5671.8
L.S.D. 0.05	0.18	0.34	3.08	3.08	185.41	257.48

Table 8 Contd.,

Season 2012						
Full dose of inorganic fertilizers	11.5	4.3	62.61	37.39	1163.12	4296.4
PGPR	10.6	3.1	70.75	29.25	1079.87	4458.8
PGPR + 1/ 4 dose of inorganic fert.	12.5	4. 7	62.40	37.60	1133.59	4194.3
PGPR + 1/ 2 dose of inorganic fert.	12.9	4.8	62.79	37.21	1164.66	4425.7
PGPR +3/ 4 dose of inorganic fert.	12.7	5.6	55.91	44.09	1231.72	5657.4
PGPR + full dose of inorganic fert.	13.6	5.9	56.62	43.38	1283.16	6287.5
L.S.D. 0.05	0.13	0.28	2.49	2.49	97.05	212.87

The highest number of female flowers per plant was reached its maximum values with combination of bio and inorganic fertilizers treatments as compared with solely treatments in both seasons of study.

Increment the numbers of female flowers with most applied treatments is a good beginning of a good harvest. Where, that will followed also by increasing of total fruits number. This means that was preceded with high percentages in setted fruits. As shown in **Table (8)** PGPR + full dose of inorganic fertilizers treatment gave the highest values of total fruits number / plant and the percentages of fruit setting as well when compared different treatments.

On the other hand, abortion of flowers was decreased since percentage of flower abscission was decreased to reach the 5% level of significance with combination of bio and inorganic fertilizers applied treatments as compared with individually treatments in both seasons. The exception was only in the case of PGPR + 1/2 dose of inorganic fertilizers treatment that insignificant decrease was recorded during 2012 season. Again, it could be concluded that reduction in flowers abscission percentages of in turn enhancement of fruit setting obtained with different PGPR in combination with inorganic fertilization treatments may be due to increase of total carbohydrates, protein and inorganic concentrations in the leaves (source) as well as the endogenous auxins, specially at full blooming and setting stages.

The different combination of bio with inorganic fertilizers treatments increased the part of assimilates being allocated to the economic part of ananas melon, (i.e., fruits) as resulted in increasing both of fruits yield / plant and weight of fruit (i.e., economic yield). Enhancing effect of such treatments on sweet ananas melon plant on yield was mainly due to their promotional effect on fruit setting and number of fruits/plant rather than fruit weight. This also could be due to the pronounced enhancable effect of the same treatments on vigorous growth behavior **Table (5)**, metabolic activity **Table (6)** (chlorophyll, carbohydrates and inorganic content) and the bioconstituents, i.e., endogenous phytohormones content **Table (7)**. All of them positively correlated with increasing fruits yield and its components **Table (8)**. In few words, data revealed that the highest yield and its components was positively corralled with the previously mentioned and discussed relations and parameters. Once again, plants of these treatments were of the highest carbohydrates content might be exported sufficient sugars at early stages, those which essentially required for fruit setting activities**.** Moreover, the inducible effect of applied treatments on fruit setting might be also due to their promotion effect on the most sensitive reproductive organs (pollen grains and ovules) and their viability, in turn, the efficiency of fertilization process and their associated hormonal stimulation**.**

In other words, These results may be due to the role of plant growth promoting rhizobacteria (PGPR) in production plant growth promoting substances (PGPs) ,tabulated in **Table (1)** that play vital role in flowering characteristics, fruit yield and its components. **Gutierrez-Manero,** ***et al.*****, (2001)** reported that several PGPR may affect plant growth through the production and release of stimulatory metabolites such as auxins and gibberellins.

Regarding the fruit quality characteristics, data in **Table (9)** obviously indicate that fruits essential bioconstituents (i.e., N, P, K, total sugars content) as well as total soluble solids (%) were affected as other mentioned results. It was clear that the applied biofertilizer a combined with inorganic fertilization treatments, obviously increased such bioconstituents compared with those of solely treatments in 2012 season.

Table 9: Effect of Inorganic and Bio – Fertilization on Fruits Quality of Sweet Ananas Melon Fruits during2012 Season

Treatments	Inorganic (Mg/G Dry Weight)			Total Soluble Solids (%)	Total Sugars
	N	P	K		
Full dose of inorganic fertilizers	53.2	61.7	38.7	12.7	93.0
PGPR	47.7	49.4	33.1	10.5	79.9
PGPR + 1/ 4 dose of inorganic fert.	50.3	53.5	41.4	10.6	89.1
PGPR + 1/ 2 dose of inorganic fert.	55.8	59.8	37.2	12.1	91.4
PGPR +3/ 4 dose of inorganic fert.	54.5	60.8	43.6	12.8	97.2
PGPR + full dose of inorganic fert.	57.6	63.3	46.0	13.4	101.7

The present findings are in accordance with those reported by **El-Kramany *et al.*, (2000), El-Habbasha *et al.*, (2007), Hosseny and Ahmed (2009), Kumar *et al.*, (2009), Boghdady *et al.*, (2012) and Dubey *et al.*, (2012).** Also, they stated that the application of biofertilizers with half dose of the recommended dose of inorganic fertilizers resulted in growth equivalent to full dose treatment of inorganic fertilizers (100% of the recommended dose) without compromising with the plant growth and yield.

The above mentioned results evidently indicated that the applied treatments were greatly increased the ability of ananas melon fruits as sink organs. So, absorption of these elements, their translocation into fruits being highly stimulated under such treatments

The obtained positive bioconstituents responses are the result of increasing leaf area and its reversion upon increasing the net photosynthesis per unit of leaf area. Also, such promotional effect of these treatments on carbohydrates and sugars concentrations in ananas melon fruits, could be due to their similar effect on chlorophyll content, number of leaves and total leaf area (surfaces of photo-assimilation), as well as their capacity of CO_2 fixation and carbohydrates synthesis compared with photosynthesis efficiency of individually treatments plants.

These results are a great interest because they mean that used treatments not only increased essential bioconstituents accumulation in fruits but also that existed in shoots. That in other meaning strictly proved that the applied treatments obviously increased the efficiency of photosynthesis in plants treated with them. In addition, this stimulation of essential bioconstituents production considered as a direct result of that vigorous growth including the photosynthetic area and photosynthetic pigments in leaves of ananas melon during different stages of growth. Applied treatments made ananas melon fruits with high nutritive value, i.e. it increased their validity for human consumption.

CONCLUSIONS AND RECOMMENDATION

Based on previous results it could be concluded that the best morphological and physiological behavior of ananas melon plant under the used treatments specially PGPR in combination with inorganic fertilization at different levels resulted in increasing fruits yield and its components, was mainly due to its simulative effect to induce best physiological and metabolical performances of ananas melon plants towards maximizing its growth and productivity. Finally it could

recommended that using of half-recommended dose of inorganic fertilizers in combination with biofertilizers to have high growth and yield of sweet ananas melon without significant differences with highest treatment (full dose of inorganic fertilizer with PGPR) and decrease the environmental pollution caused by repeated application of inorganic fertilizers at the same time.

REFERENCES

1. **A. O. A. C. (1990):** Official Method of Analysis, 10th Ed., Association of Official Analysis Chemists, Inc. USA.
2. **Abdo, F. A. (2008):** Effect of phosphate dissolving bacteria inoculation and phosphorus fertilization on growth, yield and seed quality of soybean. Egypt. J. Agric. Res., 86(3): 985-1009.
3. **Boeing, H., Bechthold, A., Bub, A., Ellinger, S., Haller, D., Kroke, A., Leschik-Bonnet, E., Müller, M.J., Oberritter, H., Schulze, M., Stehle, P., & Watzl, B. (2012).** Critical review: Vegetables and fruit in the prevention of chronic diseases. Eur. J. Nutr, 51(6), 637-663.
4. **Boghdady, M. S., Rania; M. A. N. and Fouad, A. A. (2012**): Response of sesame plant (Sesamum orientate L.) to treatments with inorganic and bio-fertilizers, Research Journal of Agriculture and Biological Sciences, 8(2): 127-137.
5. **Burns, R. G. and R. P. Dick (2002).** Enzymes in the environment-activity, ecology, and application. Marcel Dekker, Inc.
6. **Chapman, H. D.; P. F. Pratt (1961)**: *Methods of analysis for soils, plants and waters*. University of California, Berkeley, Division of Agricultural Sciences. 309 pp.
7. **Cottenie, A.; M. Verloo; L. Kiekens; G. Velgh; R. Camerlynch (1982)**: Chemical analysis of plants and soils. Laboratory of Analytical and Agrochemistry, Ghent State University: Ghent, 63 p.
8. **Deriaux, M.; Kerrest, R. and Montalon, Y.(1973):** Etude de la sulface foliare et de 1, activite photosynthetique chez qulques hybrids de mais. Ann. Amelior plantes, 23: 95-107.
9. **Dubey, R. C., Maheshwari, D. K., Varun Kumar and Pandey, R. R. (2012):** Growth enhancement of Sesamum indicum L. by rhizosphere-competent Azotobacter chroococcum AZO2 and its antagonistic activity against Macrophomina phaseolina. Phytopathology and Plant Protection, 45: 437-454.
10. **Dubois, K. A., Gilles, J. K., Ramilton, R. P. A. and Smith, F. (1956):** colorimetric method for determination sugars and related substances. Anal. Chem. 28: 350-356.
11. **El-Habbasha, S. F., Abd El-Salam, M. S. and Kabesh, M. O. (2007):** Response of two sesame varieties (Sesamum indicum L.) to partial replacement of chemical fertilizers by bio-organic fertilizers. Res. J. of Agric. And Biological Sciences, 3(6): 563-571.
12. **El-Kramany, M. F., Ahmed, M. K. A., Bahr, A. A. and Kabesh, M. O. (2000):** Utilization of biofertilizers in field crop production. 13-Effect of organic manuring, chemical and biofertilizers on yield and nutrient content of sesame grown in newly reclaimed soil. Egypt. J. Appl. Sci., 15(11): 137-155.

13. **El-Nagdy, G. A., Dalia, M. A. N., Eman A. E. and Gelan, S. A. E. (2010):** Response of flax plant (Linum usitatissimum L.) to treatments with inorganic and bio-fertilizers from nitrogen and phosphorus. Journal of American Science, 6(10): 207-217.

14. **FAO (2008)**: Guide to laboratory establishment for plant nutrient analysis. (Eds. ***M. R. Motsara and R. N. Roy***), Fertilizer and Plant Nutrition Bulletin Vol. 19: food and agriculture organization of the united nations, Rome.

15. **Glick, B. R., (1995):** The enhancement of plant growth by free-living bacteria. Can. J. Microbiol., 41: 109-117.

16. **Gutierrez-Manero, F.J., Ramos, B., Probanza, A., Mehouachi, J. and Talon, M. (2001) .**The plant growth promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. Physiol. Plant, 111: 206–211.

17. **Horneck, D. A. and Hanson, D.(1967):** Determination of Potassium and sodium by flam emission Spectrophotometry. In Handbook of Reference Methods for Plant analysis. Kalra, Y. P.(ed.): 153-155.

18. **Horneck, D. A. and Miller, R. O. (1998):** Determination of total nitrogen in plant tissue. In Handbook of Reference Methods for Plant analysis. Kalra, Y. P.(ed.): 75-83

19. **Hosseny, M. H.; M. M. M Ahmed (2009**): Effect of nitrogen, organic and PGPR on productivity of lettuce (CV. Romaine) in sandy soil under Assuit conditions. Ass. Univ. Bull. Environ. Res. Vol. 12 (1): 79 – 93.

20. **Jackson, M. L. (1965)**: Soil Chemical Analysis. Prentice Hall of India Ltd. New Delhi.

21. **Jansen, R. J., Robinson, D. P., Stolzenberg-Solomon, R. Z., Bamlet, W. R., De Andrade, M., Oberg, A. L., Hammer, T. J., Rabe, K. G., Anderson, K. E., Olson, J. E., Sinha, R., & Petersen, G. M. (2011)**. Fruit and vegetable consumption is inversely associated with having pancreatic cancer. Cancer Causes and Control, 22(12), 1613-1625

22. **Kapulnik, Y., (1991):** Plant growth-promoting rhizobacteria, p. 717-729. In: Y. Waisel; A. Eshel and V. Kafkafi (eds.). Plant roots, the hidden half. Marcel Dekker, New York.

23. **Kloepper, J. W., Zablotowicz, R. M., Tipping, E. M. and Lishitz, R. (1991):** Plant growth promotion mediated by bacterial rhizosphere colonizers. In The Rhizoshere and Plant Growth. Eds. D.L. Keister and P.B. Cregan. p. 315-326. Kluwer Academic Publishers. Dordrecht. The Netherlands.

24. **Kohler, J.; F. Caravaca; L. Carrasco and A. Rolden (2007)**: Interactions between a plant growth-promoting rhizobacterium, an AM fungus and phosphate-solublizing fungus in the rhizosphere of Lactuca sativa. Appl. Soil Ecol., 35 (2007), pp. 480–487

25. **Koshioka, M.; Harda, J.; Noma, M.; Sassa, T.; Ogiama, K; Taylor, J. S.; Rood, S. B.; Legge, R. L. and Pharis, R. P. (1983):** Reversed – phase C_{18} high performance liquid chromatography of acidic and conjugated gibbberellins. J. Chromatgr., 256:101-115.

26. **Kumar, S., Pandey, P. and Maheshwari, D. K. (2009):** Reduction in dose of chemical fertilizers and growth enhancement of sesame (Sesamum indicum L.) with application of rhizospheric competent Pseudomonas aerugionsa LES4. European Journal of Soil Biology, 45: 334-340.

27. **Lazarovits, G. and Nowak, J. (1997):** Rhizobacteria for improvement of plant growth and establishment. Hort. Sci., 32(2): 188-192.

28. **Lester, G., (1997):** Melon (Cucumis melo L.) Fruit nutritional quality and health Functionality, Hort. Technology, 7: 222-227.

29. **Maheshwari, D.K. (2010.)**: Plant Growth and Health Promoting Bacteria, Microbiology Monographs Springer-Verlag Berlin Heidelberg .18, DOI 10.1007/978-3-642-13612-2_2,

30. **Majkowska-Gadomska, J., (2009)**: Inorganic content of melon fruit (Cucumis melo L.), PhD, Chair of Horticulture, University of Warmia and Mazury in Olsztyn, J. Elementol., 14(4): 717-727.

31. **Mandal, A.; A. K. Patra, D. Singh, A. Swarup, Masto, R. E. (2007)**: Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. *Bioresource Technology*, Vol. 98: 3585–3592. doi:10.1016/j.biortech.2006.11.027.

32. **Nicander, B.; Stahl, U.; Bjorkman, P. and Tillberg, E. (1993):** Immyno affinity co-purification of cytokinins and analysis by high-performance liquid chromatography with ultra violet spectrum deterction . planta, 189: 312-320.

33. **Noel, T. C., Sheng, C., Yost, C. K., Pharis, R. P. and Hynes, M. E. (1996):** Rhizobium leguminosarum as a plant growth-promoting rhizobacterium direct growth promotion of canola and lettuce. Can. J. of Microbiol., 42: 279-283.

34. **Parham, J.A.; S.P. Deng and W.R. Raun (2003)**: Long-term cattle manure application in soil. Part II: effect on soil microbial populations and community structure. Biology and Fertility of Soils, 38 (2003), pp. 209–215

35. **Piper, G. S.(1974):** Soil and plant analysis. The Univ. of Adrlaide.

36. **Plaza, C; .D. Hernandez; J.C. Garcia-Gil and A. Polo (2004)**: Microbial activity in pig slurry-amended soils under semiarid conditions. Soil Biology & Biochemistry, 36 (2004), pp. 1577–1585

37. **Rajasekaran, S. ; K. Sankar Ganesh ; K. Jayakumar ; M. Rajesh ; C. Bhaaskaran and P. Sundaramoorthy (2012)**: Biofertilizers-Current Status of Indian Agriculture. International Journal of Environment and Bioenergy, 2012, 4(3): 176-195

38. **Saber, M. S. M. and Kabesh, M. O. (1990):** Utilization of biofertilizers in field crop production. II- A comparison study on the effect of PGPR or sulpher application on yield and nutrients uptake by lentil plants. Egypt. J. Soil Sci., 30(3): 415-422.

39. **Sandell, R.(1950):** Colorimetric determination of traces of metal 2nd Ed. Interscience publishers. Inc. New York.

40. **Schinner, F.; R. Oehlinger; E. Kandeler; R. Margesin (1997):** Methods in Soil Biology. Springer Lab Manuals, Part I. pp 213, 241.

41. **Shindy, W.W. and Smith, O.E. (1975):** Identification of plant hormones from cotton ovules. plant physiol., 55:550-554.

42. **Shindy, W.W. and Smith, O.E. (1975):** Identification of plant hormones from cotton ovules. plant physiol., 55:550-554.

43. **Snedecor, G. W. and Cochran, W. G. (1982):** Statistical Methods. The Iowa state University press, 7th Ed., 2nd. Printing, 207 pp.

44. **Wareing, P. E. and Philips, I. D. J. (1981):** Growth and differentiation in plants. 3rd Ed., Programon Press, Oxford.

45. **Wettstein, D. (1957):** Chlorophyll, letal und der submicroskpische formmech sell-der-plastiden", Exptl. Cell. Res.,12:427.